

Supplementary Material to

Phenotypic evaluation of natural and industrial *Saccharomyces* yeasts for different traits desirable in industrial bioethanol production

Vaskar Mukherjee^{1,3#}, Jan Steensels^{2#}, Bart Lievens¹, Ilse Van de Voorde³, Alex Verplaetse³, Guido Aerts³,
Kris A. Willems¹, Johan M. Thevelein⁴, Kevin J. Verstrepen² and Stefan Ruyters^{1*}

¹ KU Leuven, Laboratory for Process Microbial Ecology and Bioinspirational Management, Cluster for Bioengineering Technology (CBET), Department of Microbial and Molecular Systems (M2S), Campus De Nayer, Fortsesteenweg 30A, B-2860 Sint-Katelijne-Waver, Belgium

² KU Leuven, Laboratory for Genetics and Genomics & VIB Laboratory for Systems Biology, Centre of Microbial and Plant Genetics (CMPG), Department of Microbial and Molecular Systems (M2S), Gaston Geenslaan 1, B-3001 Leuven, Belgium

³ KU Leuven, Laboratory of Enzyme, Fermentation, and Brewing Technology, Cluster for Bioengineering Technology (CBET), Department of Microbial and Molecular Systems (M2S), Campus KaHo Sint-Lieven, Gebroeders De Smetstraat 1, B-9000 Ghent, Belgium

⁴ KU Leuven, Laboratory of Molecular Cell Biology, Institute of Botany and Microbiology, and VIB Department of Molecular Microbiology, Kasteelpark Arenberg 31, B-3001 Leuven, Belgium

*corresponding author:

stefan.ruyters@kuleuven.be

Fortsesteenweg 30A, B-2860 Sint-Katelijne-Waver, Belgium

Tel +32 15 30 55 97

Fax +32 15 30 55 99

#These authors contributed equally

Key words: bioethanol, fermentation, high-throughput, phenotype, *Saccharomyces spp.*, stress tolerance

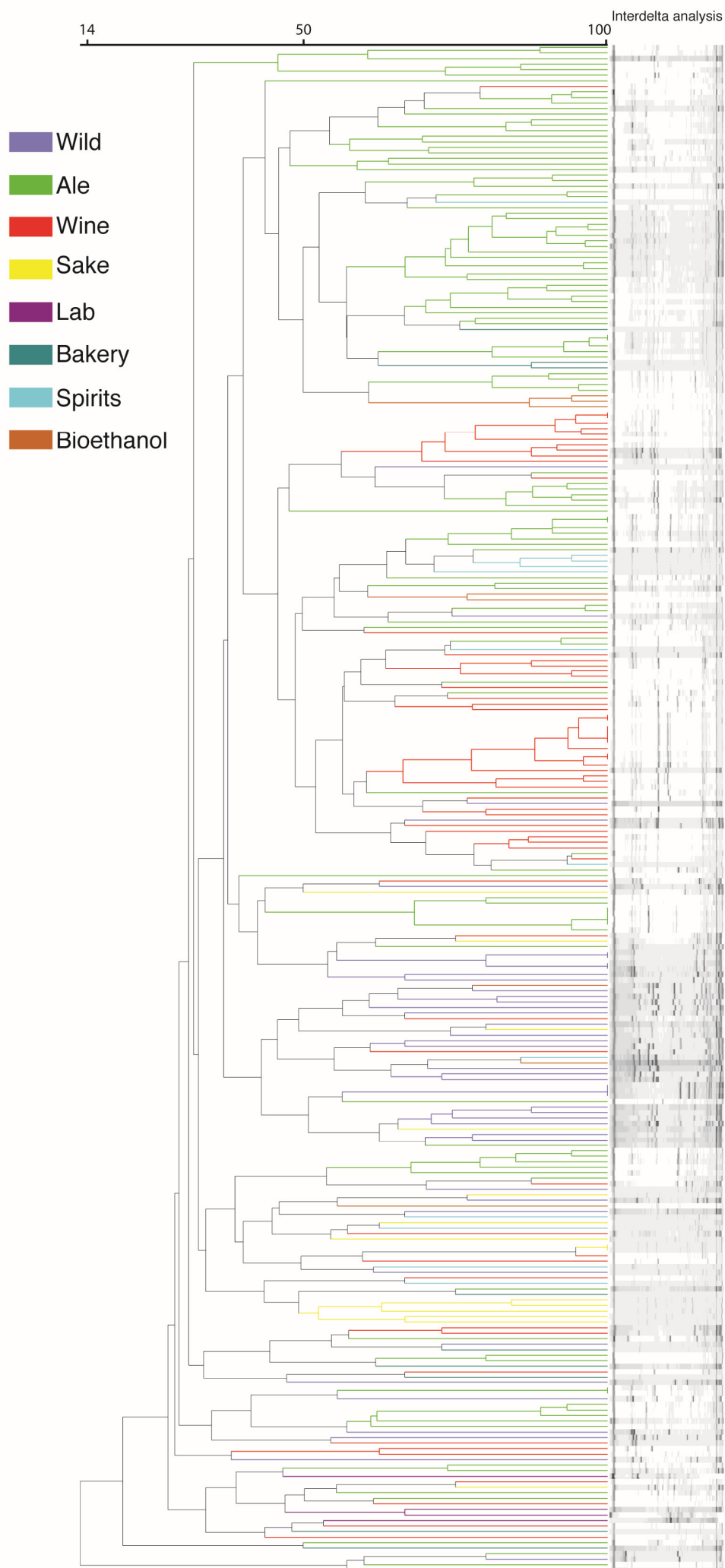
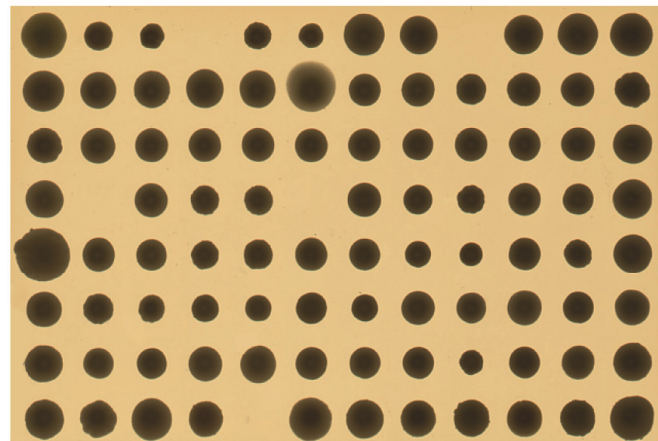
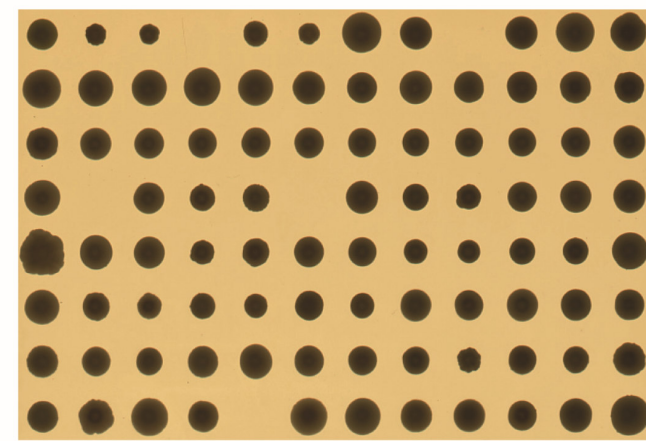


Figure S1: Genotypic clustering of the *Saccharomyces cerevisiae* strains investigated in this study. Strains from different origins are marked in different colors (see Figure for color legend). Fingerprints for each strain are given.

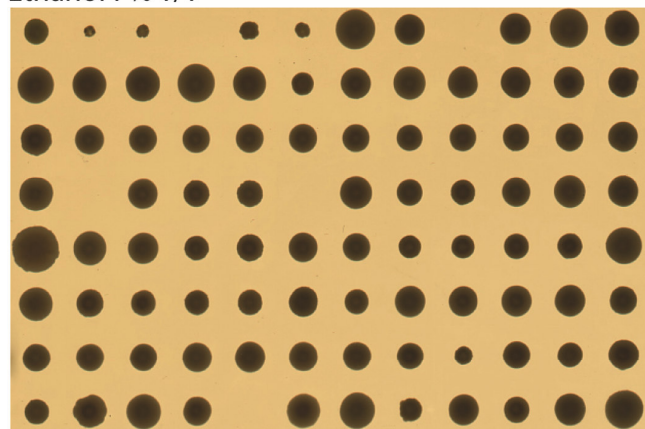
Control Plate (No Ethanol)



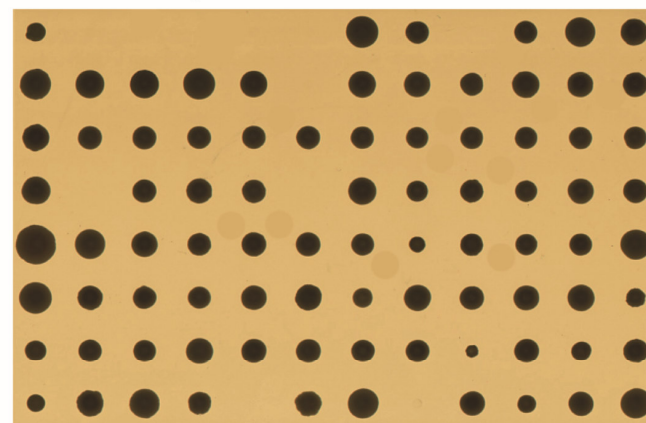
Ethanol 5% v/v



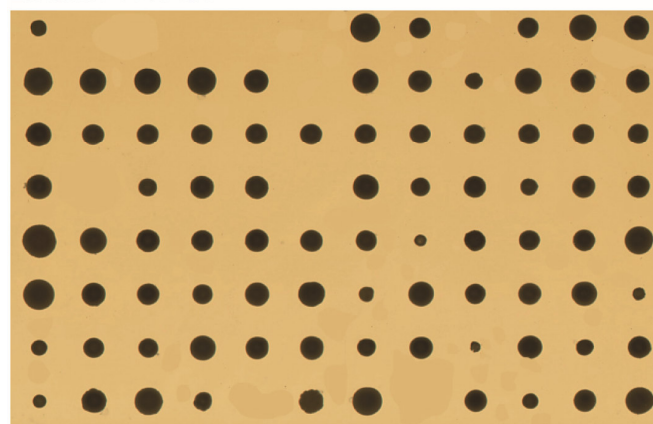
Ethanol 7% v/v



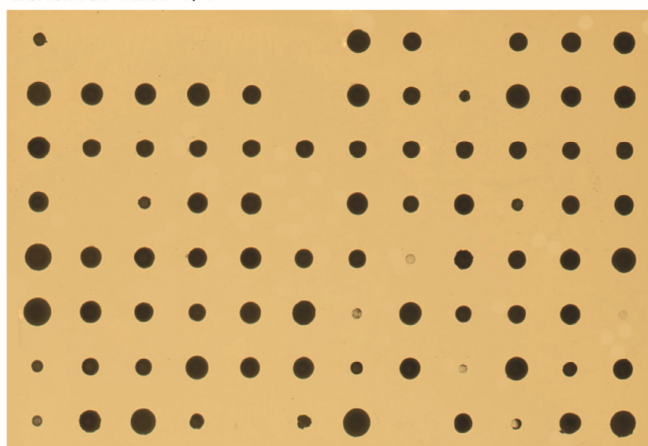
Ethanol 10% v/v



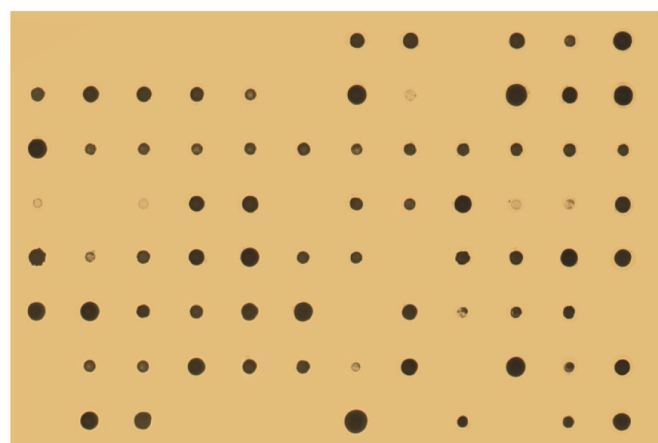
Ethanol 11% v/v



Ethanol 12% v/v



Ethanol 13% v/v



Ethanol 14% v/v

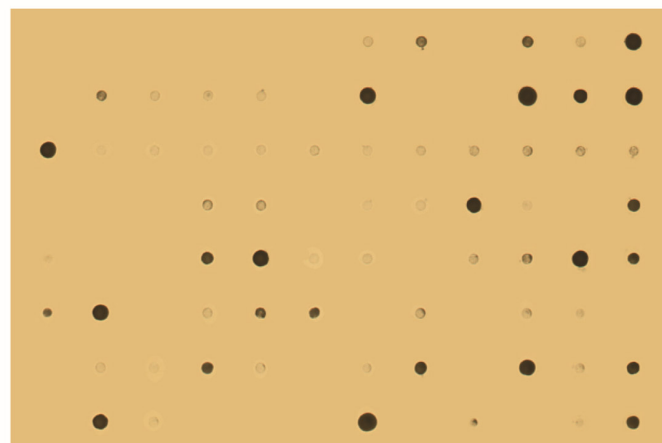


Figure S2: Phenotypic diversity of *Saccharomyces* strains on increasing concentrations of ethanol; representative images of the plates

Supplementary Table S1: Overview of the phenotypic profiling parameters and corresponding test concentrations

Osmotolerance (Tokuoka 1993; Ok and Hashinaga 1997; Tedrick et al. 2004; Dudley et al. 2005; Osho 2005; Watanabe et al. 2010)	Glucose % w/v	2	40	46	48	50	55	60	70
	Fructose % w/v	2	40	46	48	50	55	60	70
	Sorbitol % w/v	10	30	40	44	46	48	50	55
Ethanol Tolerance (Alper et al. 2006; Yoshikawa et al. 2009; Tikka et al. 2013)	Ethanol % v/v	5	7	10	11	12	13	14	15
Halotolerance (Lahav et al. 2002; Dudley et al. 2005; Yoshikawa et al. 2009)	NaCl (mM)	500	1000	1500	2000	2250	2500	2750	3000
	KCL (mM)	1000	1500	2000	2500	2750	3000	3500	4000
	LiCl (mM)	10	50	100	200	300	400	500	600
Metal Tolerance (Vadkertiová and Sláviková 2006)	Zn (mM)	1	2	3.5	5	6	7	8	10
	Cu (mM)	0.1	0.2	0.3	0.4	0.5	1	1.5	2
	Cd (mM)	0.25	0.5	1	1.5	2	2.5	3	3.5
Furan Derivative (Almeida et al. 2007; Wikandari et al. 2010;)	HMF (g/L)	2	3	4	5	6	7		
Thermotolerance (Ballesteros et al. 1991)	Incubation Temperature (°C)	24	30	37	39	41			

Supplementary Table S2: Percentage of strains growing at reference conditions and the corresponding coefficient of variations.

Reference conditions	Glucose 48%	Fructose 48%	Sorbitol 44%	NaCl 1000 mM	KCl 1500 mM	LiCl 50 mM	ET 12%	T 39°C	HMF 3g/L	ZnCl ₂ 5mM	CdSO ₄ 0.5mM	CuSO ₄ 0.1mM
% of strain growing	52	56	51	58	70	47	55	50	50	40	40	32
Coefficient of Variation	1.12	1.06	1.10	1.14	0.84	1.77	1.10	1.37	1.44	1.73	1.74	1.74

Supplementary Table S3: Relative growth of all strains on all parameters (see Table S1), except for parameters at which no growth of any of the strains was recorded.

See [xlsx database in the other supplementary file](#)

Supplementary Table S4A: Pairwise comparisons of different species using Wilcoxon rank sum test with holms correction of p value

Comparing species														
	1	2	3			1	2	3			1	2	3	
2	0.0022	-	-	Glucose	2	0.0356	-	-	Fructose	2	0.00045	-	-	Sorbitol
3	0.8167	0.1624	-		3	0.7806	0.7806	-		3	0.36778	0.01855	-	
4	9.70E-06	9.80E-08	0.0041		4	1.10E-05	5.90E-07	0.0018		4	4.70E-07	4.50E-10	0.0335	
	1	2	3			1	2	3			1	2	3	
2	0.02	-	-	NaCl	2	0.0058	-	-	KCl	2	0.032	-	-	LiCl
3	0.47	0.97	-		3	0.1415	0.0078	-		3	0.891	0.491	-	
4	0.97	0.64	0.97		4	5.40E-05	2.30E-06	0.564		4	0.119	0.856	0.792	
	1	2	3			1	2	3			1	2	3	
2	0.01763	-	-	Ethanol	2	1.00E-08	-	-	Temp	2	0.51	-	-	HMF
3	0.19414	0.01763	-		3	0.56509	0.00086	-		3	1	1	-	
4	2.00E-07	0.01763	0.00036		4	1.20E-05	0.24307	0.11076		4	5.00E-09	6.30E-10	3.90E-05	
	1	2	3			1	2	3			1	2	3	
2	1	-	-	Zn	2	3.50E-13	-	-	Cd	2	0.0061	-	-	Cu
3	0.328	0.372	-		3	0.7847	0.0007	-		3	0.0223	7.70E-05	-	
4	0.016	0.149	1		4	0.9212	8.70E-09	0.9212		4	0.0024	0.9666	4.10E-05	
1 <i>S. cerevisiae</i> ; 2 <i>S. paradoxus</i> ; 3 <i>S. bayanus</i> ; 4 <i>S. pastorianus</i>														

Supplementary Table S4B: Pairwise comparisons of different origins of *S. cerevisiae* strains using Wilcoxon rank sum test with holms correction of p value

	1	2	3	4	5	6			1	2	3	4	5	6	
2	1	-	-	-	-	-	Glucose	2	1	-	-	-	-	-	Fructose
3	2.10E-05	0.067	-	-	-	-		3	0.00126	0.04992	-	-	-	-	
4	0.933	1	0.067	-	-	-		4	1	1	0.01883	-	-	-	
5	4.90E-09	0.609	0.205	0.266	-	-		5	0.00351	1	0.27182	0.24751	-	-	
6	2.10E-06	0.146	1	0.036	1	-		6	0.00071	0.27182	1	0.01151	0.86798	-	
7	2.40E-09	0.203	1	0.073	0.609	1		7	1.30E-07	0.14604	1	0.00782	0.06658	1	
	1	2	3	4	5	6			1	2	3	4	5	6	
2	1	-	-	-	-	-	Sorbitol	2	0.617	-	-	-	-	-	NaCl
3	0.00021	0.01479	-	-	-	-		3	1	1	-	-	-	-	
4	1	1	0.03875	-	-	-		4	0.025	1	0.568	-	-	-	
5	0.15443	1	0.01632	1	-	-		5	0.556	1	1	0.138	-	-	
6	0.00021	0.01217	1	0.03717	0.07408	-		6	1	0.253	1	0.042	1	-	
7	4.50E-06	0.2047	1	0.12522	0.03717	1		7	1	1	1	0.188	1	1	
	1	2	3	4	5	6			1	2	3	4	5	6	
2	1	-	-	-	-	-	KCl	2	0.19	-	-	-	-	-	LiCl
3	0.1839	1	-	-	-	-		3	1	1	-	-	-	-	
4	0.3797	0.6342	0.0803	-	-	-		4	0.02	1	0.68	-	-	-	
5	0.2473	1	0.2128	1	-	-		5	0.13	1	1	0.37	-	-	
6	0.1379	1	1	0.0501	0.1191	-		6	1	1	1	0.15	1	-	
7	7.30E-05	1	1	0.0068	0.0016	1		7	0.68	1	1	1	1	1	
	1	2	3	4	5	6			1	2	3	4	5	6	
2	0.0055	-	-	-	-	-	Ethanol	2	0.076	-	-	-	-	-	Temp
3	2.80E-06	0.0018	-	-	-	-		3	6.10E-05	0.449	-	-	-	-	
4	3.00E-	1	0.0331	-	-	-		4	0.065	1	1	-	-	-	

	05															
5	2.00E-16	1	0.0018	1	-	-		5	2.10E-12	1	1	1	-	-		
6	3.00E-05	1	0.006	1	1	-		6	8.70E-07	1	1	0.308	0.185	-		
7	4.30E-06	1	0.0428	1	1	1		7	5.20E-09	1	1	1	1	1		
	1	2	3	4	5	6		1	2	3	4	5	6			
2	1	-	-	-	-	-	HMF	2	0.99	-	-	-	-	-	Zn	
3	0.00405	1	-	-	-	-		3	1	1	-	-	-	-		
4	1	1	0.05708	-	-	-		4	1	1	1	-	-	-		
5	2.00E-16	0.08585	1	0.00015	-	-		5	0.5	1	1	1	-	-		
6	0.001	1	1	0.05419	0.58708	-		6	1	1	1	1	1	-		
7	2.50E-11	0.31376	1	0.00258	1	1		7	1	0.52	1	1	0.99	1		
	1	2	3	4	5	6		1	2	3	4	5	6			
2	1	-	-	-	-	-	Cd	2	0.26188	-	-	-	-	-	Cu	
3	0.709	1	-	-	-	-		3	1	0.32931	-	-	-	-		
4	1	0.362	0.221	-	-	-		4	1	0.55665	1	-	-	-		
5	1	1	1	0.362	-	-		5	8.10E-08	1	0.05756	0.05392	-	-		
6	1	1	1	1	1	-		6	0.66939	0.03888	1	0.32931	0.00107	-		
7	0.043	1	1	0.092	1	1		7	1	0.25744	1	1	0.00033	1		
1= Ale; 2= Bakery; 3= Biofuel; 4= Sake; 5= Wine; 6= Spirit; 7= Wild																

References

- Almeida JRM, Modig T, Petersson A, Hahn-Hägerdal B, Lidén G, Gorwa-Grauslund MF (2007) Increased tolerance and conversion of inhibitors in lignocellulosic hydrolysates by *Saccharomyces cerevisiae*. J Chem Technol Biotechnol 82:340-349 doi:10.1002/jctb.1676
- Alper H, Moxley J, Nevoigt E, Fink GR, Stephanopoulos G (2006) Engineering yeast transcription machinery for improved ethanol tolerance and production. Science 314:1565-1568 doi:10.1126/science.1131969
- Babazadeh R, Adiels CB, Smedh M, Petelenz-Kurdiel E, Goksör M, Hohmann S (2013) Osmostress-induced cell volume loss delays yeast Hog1 signaling by limiting diffusion processes and by Hog1-specific effects. PLoS ONE 8:e80901 doi:10.1371/journal.pone.0080901
- Ballesteros I, Ballesteros M, Cabanas A, Carrasco J, Martin C, Negro MJ, Saez F, Saez R (1991) Selection of thermotolerant yeasts for simultaneous saccharification and fermentation (SSF) of cellulose to ethanol. Appl Biochem Biotechnol 28(9):307-315 doi:10.1007/bf02922610
- Dudley AM, Janse DM, Tanay A, Shamir R, Church GM (2005) A global view of pleiotropy and phenotypically derived gene function in yeast. Mol Syst Biol 1:2005.0001 doi:10.1038/msb4100004
- Lahav R, Fareleira P, Nejdat A, Abeliovich A (2002) The identification and characterization of osmotolerant yeast isolates from chemical wastewater evaporation ponds. Microb Ecol 43:388-396 doi:10.1007/s00248-002-2001-4
- Tedrick K, Trischuk T, Lehner R, Eitzen G (2004) Enhanced membrane fusion in sterol-enriched vacuoles bypasses the Vrp1p requirement. Mol Biol Cell 15:4609-4621 doi:10.1091/mbc.E04-03-0194
- Ok T, Hashinaga F (1997) Identification of sugar-tolerant yeasts isolated from high-sugar fermented vegetable extracts. J Gen Appl Microbiol 43:39-47 doi:10.2323/jgam.43.39
- Osho A (2005) Ethanol and sugar tolerance of wine yeasts isolated from fermenting cashew apple juice. African J Biotechnol 4:660-662
- Tikka C, Osuru HP, Atluri N, Raghavulu PC, Yellapu NK, Mannur IS, Prasad UV, Aluru S, K NV, Bhaskar M (2013) Isolation and characterization of ethanol tolerant yeast strains. Bioinformation 9(8): 421-425 ISSN 0973-2063
- Tokuoka K (1993) Sugar- and salt-tolerant yeasts. J Appl Bacteriol 74:101-110 doi:10.1111/j.1365-2672.1993.tb03002.x
- Vadkertiová R, Sláviková E (2006) Metal tolerance of yeasts isolated from water, soil and plant environments. J Basic Microbiol 46:145-152 doi:10.1002/jobm.200510609
- Watanabe T, Srichuwong S, Arakane M, Tamiya S, Yoshinaga M, Watanabe I, Yamamoto M, Ando A, Tokuyasu K, Nakamura T (2010) Selection of stress-tolerant yeasts for simultaneous saccharification and fermentation (SSF) of very high gravity (VHG) potato mash to ethanol. Bioresour Technol 101:9710-9714 doi:10.1016/j.biortech.2010.07.079
- Wikandari R, Millati R, Syamsiyah S, Muriana R, Ayuningsih Y (2010) Effect of furfural, hydroxymethylfurfural and acetic acid on indigenous microbial isolate for bioethanol production. Agricult J 5(2):105-109 doi: 10.3923/aj.2010.105.109

Yoshikawa K, Tanaka T, Furusawa C, Nagahisa K, Hirasawa T, Shimizu H (2009) Comprehensive phenotypic analysis for identification of genes affecting growth under ethanol stress in *Saccharomyces cerevisiae*. FEMS Yeast Res 9:32-44 doi:10.1111/j.1567-1364.2008.00456.x